INADEQUACY OF SELECTIVE PLATING MEDIA IN FIELD DETECTION OF SALMONELLA

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SUMMARY

***Salmonella* is an important human enteropathogen associated with poultry. Poultry is routinely analyzed for *Salmonella* by industry, regulatory, and research laboratories. Effectiveness of selective plating media is critical to *Salmonella* laboratory analysis. Many media routinely used in the food microbiology laboratory were developed for all Gram-negative human enteric pathogens encountered in a clinical laboratory, rather than for *Salmonella* alone. The presence of extraneous, non-*Salmonella* bacteria make the recognition of *Salmonella* on these plates very difficult. This, more than any other factor, contributes to poor performance of a medium. Primarily because of the poor performance of many selective agars in detecting *Salmonella* from non-clinical samples, food microbiology laboratories usually use two or more plating media to reduce false negative results.**

Key words: Bacteria, carcass rinse, media

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DESCRIPTION OF PROBLEM

The laboratory isolation of *Salmonella* began approximately a century ago. Since that time there have been numerous reports in the scientific literature comparing the efficacy of various media and incubation conditions. An important part of the *Salmonella* laboratory procedure is the selective plating agar. Many of the currently used media were developed for the clinical microbiologist, and clinical samples have been used for the initial evaluations of these media [1]. Although these plating media were not initially developed for isolating *Salmonella* from environmental sources (and more specifically, poultry sources), several published reports have evaluated selective plating media efficacy with poultry samples [2, 3, 4, 5]. However, the conflicting results of these past studies render them of limited value to the *Salmonella* researcher. It is rare to find any two papers that recommend the same plating medium on the basis of efficacy. One report may indicate that a certain plating medium is best, and another will report that the same plating medium was the poorest performer of those tested. Therefore, the objective of this paper is not to compare media, but rather to demonstrate the inability of many commonly

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used plating media to adequately detect naturally occurring and artificially inoculated *Salmonella* from poultry carcass rinses. Food and poultry microbiologists need to be aware of the inherent error and limitations of their laboratory analyses of various food products and the primary reasons for these limitations.

Materials and Methods

SAMPLES WITH NATURALLY OCCURRING *SALMONELLA*

A sample of 24 broiler carcasses was collected directly from the end of the chill tank of a commercial processing plant. Each carcass was placed in a separate sterile plastic bag, 100 mL of sterile distilled water was added [6], and the contents were vigorously shaken for 1 min in a 1-ft arc [4]. The resulting rinse fluid was then poured into sterile specimen cups, packed in crushed ice, and transported to the laboratory. Concentrated (10X) Universal Pre-enrichment broth (UP; Difco, Sparks, MD) was added to the rinse fluid, yielding a single-strength UP. The UP broth tubes were incubated at 35°C for 24 hr, then 0.1 mL of UP was aseptically transferred to TT Hajna broth (Difco, Sparks, MD), which was incubated at 42°C for 24 hr. Next, one of each of the selective plates used in this study (Table 1) was streaked to produce isolated colony-forming units. All plates were made fresh and stored at 4°C until use. After 24 hr incubation of the plates at 37°C, three different typical *Salmonella* colony-forming units were picked from each type of media and used to inoculate Triple Sugar Iron (TSI) and Lysine Iron (LIA) agar (Difco, Sparks, MD) slants. Cultures giving typical reactions in the TSI and LIA tubes were confirmed to be *Salmonella* using *Salmonella* Poly O and Poly H serology (Difco, Sparks, MD).

SAMPLES WITH ARTIFICIALLY INOCULATED *SALMONELLA*

Approximately 10^6 cells of *Salmonella typhimurium* were inoculated into 100 mL of chicken rinse fluid (which provided a mixed background microflora), thoroughly agitated, and then streaked onto four different selective agar plates (BGS, MLIA, RP, and XLD) to produce isolated colony-forming units. After streaking, the plates were incubated at 37°C for 24 hr. Three separate colony-forming units typical of *Salmonella* were selected from each plate, biochemically characterized, and serologically confirmed to be the inoculated *Salmonella typhimurium*.

TABLE 1. Ability of seven different selective plating media to detect the presence of naturally occurring salmonellae from processed broiler carcass rinse samples

SELECTIVE PLATING MEDIA USED	SALMONELLAE-POSITIVE ISOLATIONS[A]	
	#	%
BG sulfa (BGS)[B]	17	89
Bismuth sulfite (BS)[B]	1	5
Hektoen enteric (HE)[B]	11	58
Modified lysine iron (MLIA)[C]	17	89
Rambach plus (RP)[C]	16	84
Xylose lysine desoxycholate (XLD)[B]	6	32
Xylose lysine T4 (XLT4)[C]	11	58

[A]N = 24.

[B]Difco Laboratories, Division of Becton Dickinson and Co., Sparks, MD.

[C]Unipath Ltd., Basingstoke, UK.

Results and Discussion

The ability of the seven different selective plating media to detect naturally occurring salmonellae from processed broiler carcass rinses is presented in Table 1. Nineteen out of 24 (79%) broiler carcass rinse samples were confirmed to be *Salmonella* positive with one or more of the plating agars (data not shown in table). BGS, MLIA, and RP came the closest to allowing detection of these 19 naturally contaminated *Salmonella*-positive samples. Any two of these three plating media would have detected 18 of the 19 positive samples. Two other plating media (HE, XLT4) displayed intermediate performance, allowing detection of these naturally occurring *Salmonella*, while XLD and BS performed very poorly. Other studies [7, 8, 9] also reported that HE and XLD were less effective than other media tested, although a recent survey found that both of these plating media are commonly used [9]. All seven plating agars were streaked from the same selective enrichment tubes, therefore each plate had

approximately the same number of *Salmonella* cells deposited on the agar surface. In spite of that, only one rinse sample was found to be *Salmonella* positive with all seven of the media.

Culturing of environmental samples for the presence of *Salmonella* poses several challenges to the microbiologist. To begin with, the number of viable *Salmonella* cells present in the sample taken may be very low. Worse yet, these cells may be in an injured or altered condition. But the most critical challenge of all is that these *Salmonella* cells in a sample such as chicken rinse will be in the midst of millions of other non-*Salmonella* (extraneous) bacteria. Detecting their presence thus can be very difficult. Most selective media were not developed for *Salmonella* alone, but rather for all Gram-negative human enteric pathogens encountered in a clinical laboratory.

A plating medium may perform adequately in a clinical laboratory, yet not detect *Salmonella* from environmental samples. To further dramatize how *Salmonella* cells or colony-forming units can be present on a plating medium and not be recognized, chicken rinse fluid samples (100 mL) were inoculated with 1 million *Salmonella* cells and then streaked onto four of the plating media (BGS, MLIA, RP, or XLD). The results are presented in Table 2. Even though hundreds of *Salmonella* cells (approximately 300) were deposited on each of these media, only RP detected the inoculated *Salmonella* in all eight spiked rinse samples.

Since large numbers of samples prohibit the microbiologist from selecting more than 2 or 3 CFU/plate, *Salmonella* colonies must not only be present, but present in a recognizable form. Many of the media rely on color patterns based on a pH indicator, usually a fermentable carbohydrate such as lactose. The non-*Salmonella* or extraneous bacteria such as coliforms or *Escherichia coli* will ferment the lactose and produce a color determined by the indicator. *Salmonella* does not normally ferment lactose, so its colonies' appearance will be considerably different on the media. However, abundance of these extraneous microorganisms can color an entire section of the plate surface, thereby causing the outnumbered *Salmonella* to go unnoticed. Mainly because of the poor performance of many selective agars in detecting *Salmonella* from environmental samples, microbiology laboratories usually use two or more plating media to minimize false negative results.

TABLE 2. Recovery of artificially inoculated Salmonella (106 cells) from chicken rinse fluid

SELECTIVE PLATING MEDIA USED	NUMBER OF SALMONELLA ISOLATIONS
Rambach plus (RP)[A]	8[B]
Modified lysine iron (MLIA)[A]	6
BG sulfa (BGS)[C]	3
Xylose lysine desoxycholate (XLD)[C]	2

[A]Unipath Ltd., Basingstoke, UK.

[B]N = 8 spiked carcass rinse samples.

[C]Difco Laboratories, Division of Becton Dickinson and Co., Sparks, MD.

CONCLUSIONS AND APPLICATIONS

1. *Salmonella* may go undetected in environmental (poultry) samples because of the presence of extraneous or background types of bacteria.
2. The inherent fallibility in laboratory methods must be considered when analyzing data resulting from *Salmonella* analysis.
3. Even when two laboratories are using the identical procedures, variation in the results should be expected.
4. Although none of the plating media tested in this study performed perfectly, some of these media do perform better than others.

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